

Immunofluorescence and microscopy

Alice H Huang

Updated date: Jan 11, 2022

An abbreviated version of this protocol was published in eLIFE in Jun 2020

Tgfb signaling is required for tenocyte recruitment and functional neonatal tendon regeneration

DOI: 10.7554/eLife.51779

Detailed protocol

Antibody Staining Protocols

Anti-Mouse Antibodies Staining Procedure

(using Vector Mouse On Mouse kit - Cat #: BMK-2202)

Is your target protein a transcription factor or do you have reason to believe that it's in the nucleus?

Then it is highly recommended that you use PBS with 0.1% Triton or NP-40 for all your washes and dilute your antibodies in this PBS-Triton solution. This should ensure that your antibody has better access to the nucleus than the same protocol sans Triton.

1. Wash slides 2 X 2 min. in PBS
2. Incubate 1 hr in M.O.M. Mouse Ig Blocking Reagent solution
3. Wash 2 X 2 min. in PBS
4. Incubate 5 min. M.O.M. Diluent solution
5. Pour off excess Diluent & incubate 30 min. in primary antibody (in Diluent solution)
6. Wash 2 X 2 min. in PBS
7. Incubate 10 min in M.O.M. Biotinylated Anti-Mouse IgG Reagent solution
8. Wash 2 X 2 min. in PBS
9. Incubate 5 min. in Streptavidin-Cy3 (Jackson ImmunoResearch Cat. # 016-160-084 : 15 mg/mL) or Streptavidin-C5 (Jackson Immuno) 1:200 in Diluent solution
10. Wash 2 X 5 min. in PBS
11. Mount in Gel Mount

Blocking Reagent solution: 2.5mL PBS + 2 drops of the Blocking Reagent stock

Diluent solution: 7.5mL PBS + 600 uL Diluent stock

Biotinylated Reagent solution: 2.5mL Diluent solution + 10uL Biotin Stock

Rabbit/Goat (and other non-mouse) Antibodies Staining Procedure

1. Wash slides 2 X 5 min. in PBS (can add 0.1-1% Triton X-100 or NP-40 for better penetration)
2. Block 1hr in 5% serum that secondary Ab was raised in (typically donkey or goat) in PBS
3. Incubate 3 hrs @ room temperature or overnight at 4°C in primary antibody in 5% serum that secondary Ab was raised in
4. Wash 3 X 5 min in PBS
5. Incubate 1-2 hrs in secondary Ab in 5% serum that secondary Ab was raised in
6. Wash 3 X 5 min in PBS
7. Mount in Gel Mount

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Huang, A. (2022). Immunofluorescence and microscopy. Bio-protocol Preprint. bio-protocol.org/prep1496.
2. Kaji, D. A., Howell, K. L., Balic, Z., Hubmacher, D. and Huang, A. H. (2020). Tgfb signaling is required for tenocyte recruitment and functional neonatal tendon regeneration. eLIFE. DOI: [10.7554/eLife.51779](https://doi.org/10.7554/eLife.51779)

